Phosphate-independent biodegradation of glyphosate by a native *Bacillus cereus* strain isolated from northeast Mexico

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Introduction

Glyphosate is an herbicide, which belongs to the family phosphonates[1]. These compounds are characterized by the direct covalent carbon-phosphorus (C-P) bond, which is chemically inert, resistant to hydrolysis and photolysis[2].

It is estimated that around 8.6 million tons of glyphosate have been used worldwide since its implementation in agriculture in 1976[3].

In March 2015, IARC classified glyphosate as probably carcinogenic to humans, because evidence of the adverse health effects of this herbicide[4].

Different microorganisms capable of metabolizing the phosphonate have been identified. However, no microorganisms have been identified yet capable of degrading glyphosate in the presence of phosphate[5].

Therefore, it is essential to study a glyphosate-degrading microorganism that is independent of phosphate levels in the environment[6].

Methods

1. Bioinformatic analysis of enzymes involved in the metabolism of phosphonates and polyphosphates in bacteria belonging to the family *Bacillaceae*
2. *In-vitro* assays of the metabolism of phosphonates and polyphosphates by bacteria belonging to the family *Bacillaceae*
3. Evaluation of the kinetic parameters of glyphosate metabolism supplied as the sole source of phosphorus by *Bacillus cereus* 6P.

Results and Discussion

1. Bioinformatic analysis

The presence of homologous enzymes of the catalytic component of the C-P lyase (PhnJ), phosphonoacetaldehyde hydrolase (PhnX) and the polyphosphate kinase (PPK) in bacteria belonging to the *Bacillaceae* family was determined (Table 1).

	PhnJ	PhnX	PPK
B. cereus	×	✓	✓
B. licheniformis	×	✓	×
B. thuringensis	×	✓	✓
B. subtilis	×	✓	×
B. clausi	×	✓	×

Table 1. Homologs of PhnJ, PhnX and PPK by a BlastP analysis of the amino acid sequences registered in the Genbank

2. *In-vitro* assays

The strain 6P exhibited a wide increase in the increase of total proteins, suggesting the metabolization of glyphosate. The rest of the *Bacillaceae* strains exhibited limited biomass production (Fig. 1).

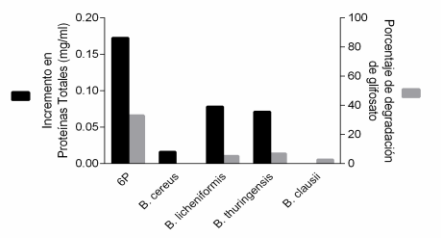

Fig 1. Biomass production and degradation of glyphosate supplied as sole source of phosphorus by bacteria of the *Bacillaceae* family.

3. Evaluation of the kinetic parameters

The biodegradation of glyphosate occurred at 100h, along with the accumulation of polyphosphate. Interestingly, the strain 6P started the exponential phase at 140h (Fig. 2).

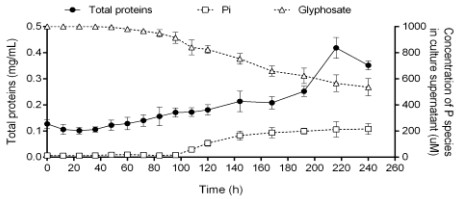

Fig. 2 Kinetics of biodegradation of glyphosate supplied as the sole source of phosphorus carried out by *Bacillus cereus* 6P.

Conclusions

- The *Bacillaceae* family does not possess the PhnJ enzyme responsible for degrading glyphosate.
- The PhnX enzyme is present in members of the *Bacillaceae* family. However, due to the behavior shown, it is not related to the degradation of glyphosate.
- *Bacillus cereus* 6P is the only bacterium of the family *Bacillaceae* that has the capacity to degrade glyphosate taking as evidence the disappearance of glyphosate in the culture medium and the increase in total proteins.
- The appearance of inorganic phosphate in the medium begins to appear in the first 100 hours of incubation as well as the accumulation of polyphosphate.

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